

# ***Helicobacter* infection: What should a swine practitioner know?**

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## **Summary**

Gastric microbiology of the pig has been given a great deal of attention in the past decade. Much of the work in this area has been published in human medical journals, and therefore, swine practitioners may not be aware of these recent findings. The pig has been used in the study of gastroduodenal ulcers of humans. The stomach of pigs has been successfully colonized experimentally by *Helicobacter pylori*, the bacterium associated with gastric ulceration and gastritis in humans. Although *H. pylori* does not naturally inhabit the pig's stomach, there are spiral-shaped bacteria that can commonly be found in the porcine gastric mucosa. This bacterium has been tentatively named *Helicobacter heilmannii* (formerly *Gastrospirillum suis*). *Helicobacter heilmannii* is found in several other species, including dogs, cats, and occasionally humans. Survey studies show that pig contact is an important risk factor for humans becoming infected with *H. heilmannii*. Infection of humans with *H. heilmannii* causes a milder form of chronic gastritis than *H. pylori* and may be self-limiting. At the present time, it is unclear whether *H. heilmannii* infection of swine causes disease. In swine, the site of ulceration is in the pars oesophagea whereas *H. heilmannii* colonizes the fundic and pyloric regions. Pigs experimentally infected have not developed gastric ulcers; however, several epidemiological studies have found a strong association between the presence of *H. heilmannii* and ulceration of the pars oesophagea. Practitioners need to consider this organism as a potential pathogen and a potential zoonotic bacterium.

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**T**he field of gastric microbiology has made rapid advances in the past decade as a result of the discovery that gastroduodenal ulceration in humans is primarily a result of a bacterial infection. Research has led to an increase in our knowledge of the curved or spiral bacteria that inhabit the mucus layer of the stomach of humans and various animal species, including swine.

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This paper is intended to provide the swine practitioner with an overview of the numerous publications on *Helicobacter* infection, with particular emphasis on its possible role in the development of gastric ulcers in swine, and its potential zoonotic spread from pigs to humans.

## **History**

The presence of spiral bacteria in the stomachs of certain animals has been recognized for over 100 years,<sup>1,2</sup> and in the stomachs of humans for at least 60 years.<sup>3</sup> Little interest was shown in these micro-organisms until the 1970s, when a link between the presence of intragastric bacteria and peptic ulcers in humans was suggested. By the early 1980s, the association between the presence of bacteria and the occurrence of chronic gastritis and peptic ulcers was established.<sup>4</sup> The species of gastric bacteria that has now become widely accepted as the major cause of chronic gastritis, gastroduodenal ulceration, and a major risk factor for gastric cancer in humans has been identified as *Helicobacter pylori*.<sup>5</sup> As a result of the discovery of a bacterial etiology, the approach to the treatment of human gastric ailments has radically changed. The treatment of gastroduodenal ulcers is accomplished with a short course of antibiotics in combination with acid-inhibiting drugs. The success rate of this regimen is very high.<sup>6</sup>

Researchers have used the pig as a model to facilitate the study of *H. pylori*. Gnotobiotic and conventional pigs have been successfully colonized with experimental infections of *H. pylori*.<sup>7</sup> In addition, a naturally occurring, spiral-shaped bacterium (morphologically distinct from *H. pylori*) has been identified in the gastric mucosa of commercially-reared pigs.<sup>8</sup>

It has become apparent that *H. pylori* is almost exclusively a human pathogen. However, a second *Helicobacter* (*H. heilmannii*) which infects humans at a much lower prevalence than *H. pylori*<sup>9</sup> is likely the same species of *Helicobacter* found in the stomachs of pigs, as well as cats and dogs. Therefore, there is a strong possibility that humans can become infected with *H. heilmannii* from pigs.<sup>10</sup>

## **Bacterial characteristics and nomenclature**

*Helicobacter* resemble *Arcobacter* and *Campylobacter* and share many characteristics. Microbiologists have grouped *Helicobacter*, *Arcobacter*, and *Campylobacter* within Superfamily VI, with *Arcobacter* and *Campylobacter* forming the family Campylobacteriaceae. Members of Superfamily VI tend to be difficult to culture and to define using conventional laboratory tests. Only recently, using advanced molecular and DNA technology, has classification been possible.<sup>11</sup>

Whether or not a bacterium belongs in the *Helicobacter* genus has been based mainly on determining the sequence of bases in its 16S ribosomal RNA molecule.<sup>12</sup> Bacteria with more than 90% homology in the sequence of bases indicates a close relationship. These criteria have been used to group bacteria with remarkably different morphological structure together in the genus *Helicobacter*.

All *Helicobacter* are gram-negative and micro-aerophilic. Commonly, they have multiple polar flagella that are always sheathed and exhibit urease, catalase, and oxidase activity.<sup>12</sup>

Two *Helicobacter* species isolated from human stomachs differ morphologically from each other. *Helicobacter pylori* (originally named *Campylobacter pyloridis*) are curved or S-shaped, with flagella at one end. They measure about 4 µm in length. The second, less prevalent organism, *Helicobacter heilmannii* (previously named *Gastrospirillum hominis*) is a tightly spiraled bacterium, with the number of turns varying between four and six. These bacteria, at 7–10 µm in length, are more than twice as large as *H. pylori*.<sup>13</sup>

The spiral bacterium found in the stomach of pigs was originally called *Gastrospirillum suis* because of the morphological similarity to the spiral bacterium “*Gastrospirillum hominis*” and other “*Gastrospirilla*” found in a variety of animal species. A comparison of the ribosomal RNA between the human and swine “*Gastrospirilla*” was shown to be 99.5% homologous, supporting the hypothesis that the bacterium of pigs and of humans belongs to the same species.<sup>14</sup> Therefore, recent studies refer to this bacterium in swine stomachs as *H. heilmannii*.

So far, more than 13 species of *Helicobacter* have been identified in a wide range of omnivorous or carnivorous animals. *Helicobacter heilmannii* and *H. felis* are the two species most often identified in dogs and cats.<sup>15</sup> Not all *Helicobacter* species are restricted to the stomach. Certain *Helicobacter* species have been identified in the lower bowel, liver, and in aborted fetuses.<sup>15</sup>

## Epidemiology

*Helicobacter pylori* infection is widespread, with one-third to one-half of the world's human population carrying the bacterium.<sup>16,17</sup> In developed countries, children are rarely infected but the prevalence rises with age, so that more than half of all 60-year-olds are infected. The incidence in developed countries is low (0%–5% per year), but once acquired, *H. pylori* infection persists for years and often the infected individual remains a carrier for life. In contrast, the prevalence of *H. pylori* in developing countries is very high (generally two-thirds of the adult population infected) and children are commonly infected.<sup>6</sup> General hygiene and sanitation are believed to be important in controlling the spread of *H. pylori*. Studies have shown that *H. pylori* exists in a higher prevalence in saliva than in feces,<sup>18</sup> suggesting that the oral-oral route of spread may be the most likely means by which *H. pylori* is transmitted in developed countries. Fecal transmission is, however, a likely method of spread when sanitation is poor. An animal reservoir as a source of *H. pylori* has not been found, and therefore, person-to-person contact is considered the primary method of transmission.

*Helicobacter heilmannii* appears to be widely distributed among cats, dogs, and pigs. In humans, *H. heilmannii* is far less prevalent than *H. pylori*. About 1% or less of the human population is believed to be infected with *H. heilmannii*.<sup>19,20</sup> Contact with animals, particularly pigs, has been shown to be a risk factor to becoming infected with *H. heilmannii*.<sup>10,19</sup> It has been suggested that *H. heilmannii* infection in humans may often be self-limiting compared to dogs, cats, and pigs, in which the bacterium appears to persist.<sup>10</sup>

The prevalence of *H. heilmannii* in the commercial pig population is unknown. In a study of 85 pigs from an Italian slaughterhouse, 9.4% of the stomachs were found to be positive for *H. heilmannii* based on histologic examination.<sup>21</sup> In an earlier study, Brazilian researchers reported a similar prevalence, identifying 10.8% of 120 pig stomachs as positive.<sup>22</sup> More sensitive tests for detecting *H. heilmannii* are being developed and it is certain that the prevalence rate will be found to be much higher than in these initial studies.<sup>23</sup>

Transmission of *H. heilmannii* is likely via oral-oral and fecal-oral route from pig to pig. Cross-species spread appears likely, and therefore, animal vectors may be important in the introduction of *H. heilmannii* to a naïve swine herd. In a recent Italian survey of wild rats, histologic evidence of a spiral bacterium morphologically similar to *H. heilmannii* was found in 23% of the animals examined.<sup>24</sup> Cats and dogs are also known carriers of *H. heilmannii* and need to be considered as potential vectors.

## Pathogenesis

The success of *Helicobacter* organisms is related both to unique maintenance factors (allowing the organism to persist in a hostile environment), and pathogenic mechanisms (leading to disruption of the gastric mucosal barrier).<sup>25</sup> The most important maintenance (virulence) factors include motility, adaptive enzymes and proteins, and the ability to adhere to gastric mucosal cells and mucus. Pathogenic mechanisms include enzymes and toxins which act as mediators of inflammation or contribute to acid/pepsin activity.<sup>25</sup> The characteristics of *Helicobacter* which make them successful colonizers of the stomach are much better understood than the factors that lead to gastritis and ulceration.<sup>26</sup>

Motility has been shown to be an essential component of *Helicobacter*'s success in colonizing the stomach. Motility is achieved by the spiral shape and the polar flagella. For an organism to survive in the stomach, the bacteria must be able to avoid being flushed away during gastric contractions and stomach emptying. *Helicobacter* are able to quickly burrow into the mucus and away from the acid environment of the stomach lumen. Research using gnotobiotic pigs has shown that a strain of *H. pylori* with poor motility was unable to colonize the pig's stomach but a highly motile strain was successful.<sup>27</sup>

Urease production also appears to be an important survival mechanism. Urease breaks down urea to produce ammonia, which in turn, helps to neutralize the stomach acid in the surrounding area. *Helicobacter* strains exhibiting weak urease activity failed to colonize in pigs that were experimentally challenged.<sup>28</sup> Once colonization has taken

place, and the bacterium is located in the more friendly alkaline environment adjacent to the epithelial surface, urease production may not be needed as an environmental modifier but may be an essential component in the pathogenic mechanism.<sup>25</sup> Catalase, another important enzyme produced by *Helicobacter*, protects the bacterium from the toxic effects of reactive oxygen metabolites formed in neutrophils from hydrogen peroxide.<sup>26</sup>

*Helicobacter* spp. can inhibit acid secretion from isolated rabbit parietal cells<sup>29</sup> and it has been suggested that specific inhibition of acid secretion may facilitate acute *H. pylori* infection. A hypochlorhydria is frequently observed in human patients recently infected with *H. pylori*.<sup>25</sup>

Colonization of *H. pylori* requires the ability of the organism to closely adhere to the gastric epithelial surface, similar to enterotoxigenic *E. coli* in the small intestine. *Helicobacter heilmannii* does not adhere to mucosal cells, but appears to rely on its very active motility<sup>30</sup> to colonize the mucus and mucosal surface.

An amazing aspect of *H. pylori* is the fact that a strong antibody response occurs as a result of infection, and yet the bacterium generally persists for years, possibly for the life of the human host. *Helicobacter pylori* is obviously capable of evading the human immune mechanisms. This evasion is partly a result of the inaccessibility and motility of the bacterium, but other factors are likely to play a role.<sup>26</sup> Typically, the inflammatory reaction occurs deep in the gastric mucosa some distance from the bacterium, which may explain how the organisms survive.

A second aspect of bacterial persistence that puzzles researchers involves nutrient sources. It is unlikely that *Helicobacter* spp. could persist for years relying on the food that the host ingests.<sup>6</sup> Therefore, some regulated interaction between the host cells and bacteria must exist. Possibly *Helicobacter* trigger inflammation as a means of acquiring nutrients. *Helicobacter pylori* initiates irritation of the epithelia with the release of urease and production of ammonia. In addition, cytotoxins have been identified which are directly associated with the degree of gastritis in gnotobiotic pigs<sup>27</sup> and in humans. *Helicobacter pylori* strains which produce a toxin inducing the formation of vacuoles in tissue cultures are 30%–40% over-represented in ulcer patients compared to those with gastritis alone.<sup>6</sup> The gene responsible for toxin production has been identified and named vacA. A second gene of *H. pylori* that is associated with pathogenicity has been sequenced and named cagA. About 50% of patients with chronic gastritis alone are infected with cagA strains of *H. pylori*, but virtually all patients with duodenal ulcers are infected with cagA strains.<sup>6</sup>

In humans, *Helicobacter* infect and inflame the tissue that becomes ulcerated and therefore one can speculate that direct insult from the bacteria leads to ulcerative lesions; at least this appears to occur in certain patients in combination with predisposing factors. In swine, ulceration is almost exclusively restricted to the nonglandular pars oesophagea region, whereas *H. heilmannii* are observed in the oxyntic and pyloric mucosa. Therefore, it has been suggested that if *Helicobacter* is partially responsible for gastric ulceration in swine,

the mechanism might be that infected stomachs secrete too much acid.<sup>13</sup> It has been suggested that hyperacid secretion due to increased gastrin release is stimulated by the ammonia produced as a result of *H. pylori* colonization.<sup>31</sup> Supporters of this theory argue that effects by the bacteria to create a neutral pH in the layer overlying the gastric epithelium interferes with the normal inhibition of gastrin release by intraluminal acid. Eradication of *H. pylori* generally results in a decrease in basal and meal-stimulated gastrin secretion.<sup>32</sup> *Helicobacter heilmannii* infections have not been as thoroughly studied as *H. pylori*, but at least one study of pigs experimentally infected showed no difference in gastric pH values before infection with *H. heilmannii* compared to after infection.<sup>33</sup>

In all likelihood, the mechanism responsible for ulceration of the pars oesophagea in pigs is very different from the pathogenesis of the human peptic ulcer, considering the major differences in gastric anatomy and physiology between the two species. Therefore, any attempt to extrapolate information from studies of *H. pylori* in humans to explain gastric ulceration in swine should be viewed with caution.

## Diagnosis

Whereas there are many methods available for diagnosing *H. pylori* infection,<sup>34,35</sup> detecting *H. heilmannii* poses some difficulty.<sup>12</sup>

In the case of *H. pylori* diagnosis in humans, tests can be divided into those requiring endoscopy to gain diagnostic material and those that are noninvasive and can be performed on saliva, serum, or breath samples.<sup>36</sup> Histologic examination of biopsy material from the antrum is commonly performed because the likelihood of finding bacteria in positive cases is high, especially if more than one sample is taken. In addition, evidence of inflammation and mucosa pathology can be assessed.<sup>34</sup>

Often a rapid urease test is performed in conjunction with histologic examination. This test is not specific to *H. pylori* because all *Helicobacter* species are potent producers of urease. The test involves placing biopsy material in a urea broth which contains a pH indicator, such as phenol red. The pH will rise if urease is present to split urea and create ammonia. If only a small number of bacteria are present in the biopsy sample, a color change may not occur, giving a false negative. The test is only 70%–90% sensitive, but it provides a rapid answer and is relatively inexpensive to perform.<sup>15</sup>

Other tests in human medicine that can be performed—using endoscopically obtained biopsy material—include polymerase chain reaction (PCR) and culture. *Helicobacters* tend to be difficult to grow, making culturing the least sensitive of these tests; as a result, this is seldom performed outside research institutes.<sup>35</sup>

*Helicobacter pylori* gastritis causes a systemic and local immune response which has been used as the basis for serological tests. Enzyme-linked immunosorbent assay (ELISA) tests are 95% sensitive and specific.<sup>36,37</sup> Antibodies remain detectable for 4–6 months after *H. pylori* eradication.

The urea breath test relies on the ability of *Helicobacter* spp. to break

down urea that is radiolabeled with C13 or C14 to produce radioactive carbon dioxide.<sup>38</sup> Exhaled air is sampled and the degree of radioactivity measured as a means of determining whether urease-producing bacteria are present in the stomach of the patient. The test is highly sensitive and specific, easy to perform, and produces a rapid result. The breath test is commonly used in human medicine as a follow-up method of monitoring the success of *H. pylori* eradication programs<sup>39</sup> and can be used as a screening procedure to reduce the necessity of invasive techniques.<sup>40</sup>

Diagnosing *H. beilmannii* infection in swine has proven more difficult. In situ mapping of urease-positive areas is a very useful technique that can help detect this pathogen.<sup>41</sup> This method involves collecting pig stomachs at the abattoir, opening and gently washing the mucosal surface, and painting a thin layer of a urea medium containing a pH indicator (phenol red) over the entire inner stomach surface. Within 2 hours, a striking color change from yellow to pink indicates areas of urease-producing bacterial colonies. Distribution of *H. beilmannii* colonies has been reported to be patchy, resulting in false negative results from biopsy examination. The use of a urea mapping technique helps to overcome this problem. It can also be used as an indirect method of monitoring for the presence of *H. beilmannii* on a herd basis.

Culturing of *H. beilmannii* has generally been unsuccessful.<sup>41,42</sup> Frustrated by an inability to grow non-*H. pylori* spiral bacteria in laboratory media, researchers looked for alternative methods of culture. Mice have been inoculated with gastric material and *H. beilmannii* successfully colonized.<sup>43</sup> In a recent study,<sup>23</sup> mouse inoculation was compared to histologic examination of carbolfuchsin-stained slides. Of 70 pig stomachs examined, 54 were positive using the mouse inoculation technique versus only 17 of the 70 stomachs examined by histology, and only 14 of 70 positive using the rapid urease test.

*Helicobacter* are not easily demonstrated using hematoxylin-and-eosin stain on histologic samples. To make *Helicobacter* organisms more prominent, the Warthin-Starry silver stain is favored by many researchers,<sup>34</sup> but this procedure is more difficult and more expensive than many other staining techniques, such as carbolfuchsin staining.<sup>44</sup> Other possible procedures include Giemsa stain, acridine orange (which requires a fluorescent microscope), and phase-contrast microscopic techniques.

There is a need for serological tests that would enable rapid screening of pig herds for *H. beilmannii*. In addition, culture techniques and PCR tests need to be developed.

## Clinical disease

The strong association between *H. pylori* and gastritis, gastroduodenal ulcers, and gastric cancer is well established in human medicine.<sup>6</sup> A matched control study has compared *H. pylori* and *H. beilmannii* gastritis in humans.<sup>45</sup> These researchers found that *H. beilmannii* colonization was mainly focal and restricted to the antrum, compared to a more diffuse pattern for *H. pylori*. Gastritis was significantly milder in *H. beilmannii* compared to *H. pylori*-infected patients.

Gnotobiotic pigs experimentally infected with *H. pylori* do develop gastritis, which can lead to erosions and ulceration in the glandular region of the stomach, generally in the junction of the fundus and antrum.<sup>46</sup> The inflammatory response to *H. beilmannii* is similar to *H. pylori*, except that the inflammation is distributed mainly in the fundus compared to the cardia and antrum of *H. pylori*-infected pigs. The vast majority of stomach ulcers in commercially reared swine occur in the nonglandular pars oesophageal region, an area not suitable for *Helicobacter* colonization. The pars oesophagea is lined by stratified squamous epithelial cells and is normally inhabited by various bacteria, including *Lactobacillus* and *Bacillus* organisms.

In a recent study,<sup>33</sup> gnotobiotic pigs were experimentally infected with either fermentative bacteria such as *Lactobacillus* and *Bacillus*, or *H. beilmannii*, and both groups were fed a carbohydrate-enriched diet. Piglets infected with *H. beilmannii* did not develop lesions in the pars oesophagea, but the group infected with the fermentative bacteria did develop epithelial damage in the pars oesophagea. The authors suggested that the organic acids produced by bacteria such as *Lactobacillus* and *Bacillus* and potentiated by high dietary carbohydrate levels are important interactive factors for ulcer development in the pars oesophagea.

On the other hand, the presence of *H. beilmannii* in the glandular stomach has been found to be associated with ulceration of the pars oesophagea in several epidemiological surveys.<sup>21,23,47,48</sup> Barbosa, et al.,<sup>47</sup> examined 32 pigs with grossly normal mucosa and 32 pigs with chronic ulceration of the pars oesophagea. Forty pigs (62.5%) were positive for *H. beilmannii*, and of these positive animals, 67.5% had ulcers and only 32.5% were normal. Of the 24 pigs negative for *Helicobacter*, five (20.8%) had ulceration and 19 (79.2%) were normal. Queiroz, et al.,<sup>23</sup> examined 20 pig stomachs with ulcers, 30 stomachs with parakeratosis lesions, and 20 normal stomachs. *Helicobacter* were present in 100% of stomachs with ulcers, and 90% of stomachs with parakeratosis, but only 35% of macroscopically normal stomachs. These studies suggest a strong relationship between *Helicobacter* infection and gastric ulceration.

## Zoonotic potential

Molecular biological techniques have led to the conclusion that the *H. beilmannii* organism isolated from a small proportion of human gastritis patients is the same *Helicobacter* as the organism widely distributed in the world swine population. The potential for pig-to-human spread has been suggested.<sup>45,49</sup> A recent survey of 177 patients with *H. beilmannii* infection found that *H. beilmannii* infection was strongly associated with contact with dogs, cats, cattle, or pigs.<sup>10</sup> Contact with pigs was found to be a greater risk factor than contact with other animal species. People with pig contact were almost five times more likely to be infected with *H. beilmannii* than those without pig contact.

More studies must be performed to fully investigate the relationship between *H. beilmannii* infection in pigs and humans, and to determine how the organism might be transmitted. In addition, the infection in humans and in pigs needs to be investigated to determine the

pathogenic significance of *H. heilmannii* in both species.

## Implications

- *Helicobacter pylori* causes gastroduodenal (peptic) ulcer disease in humans.
- *Helicobacter* organisms (*H. heilmannii*) commonly infect swine and may infrequently infect humans, causing a mild fundic gastritis.
- The common gastric bacterium of humans (*H. pylori*) does not naturally infect pigs but can be used to colonize the pig's stomach experimentally.
- The presence of *Helicobacter* can be detected by using in situ urease mapping and histologic examination with Warthin-Starry Silver stain.
- Experimentally infected pigs have not developed gastric ulcers; however, epidemiological studies have found a strong correlation between the presence of *H. heilmannii* and ulceration of the pars oesophagea.
- *H. heilmannii* colonizes the fundic and/or pyloric regions of the stomach, far removed from the pars oesophagea, the ulcer site in pigs, and therefore it is difficult to explain how the lesion could be caused by bacterial infection.

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